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10/7/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

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IMMUNOHISTOCHEMICAL AND PHARMACOKINETIC CHARACTERIZATION OF THE SITE-SPECIFIC IMMUNOCONJUGATE CYT-356 DERIVED FROM ANTIPROSTATE MONOCLONAL ANTIBODY 7E11-C5
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ABSTRACT: In this study, a site-specific immunoconjugate, designated CYT-356, of the prostate-reactive monoclonal antibody 7E11-C5 was characterized by immunohistological methods for reactivity with normal and neoplastic human tissues. In addition, CYT-356 labeled with 111In was assessed by in vivo imaging and pharmacokinetic studies for localization to human tumor xenografts in nude mice. The native antibody and the site-specific immunoconjugate exhibited similar patterns of reactivity with normal human tissues. Although the majority of tissues tested were negative, weak reactivity with cardiac muscle, proximal kidney tubules, and sweat glands was observed. Positive staining of normal prostate epithelial cells and glandular lumina and strong reactivity with a subset of skeletal muscle cells were also observed. CYT-356 reacted with 100% of prostate tumors examined but was negative on a variety of other neoplasms. Following i.v. administration, CYT-356-111In rapidly localized to and imaged LNCaP human prostate adenocarcinoma xenografts in nude mice, reaching maximal levels of about 30% of injected dose/g of tumor within 3 days. No unusual localization was seen to any nontumor tissue or organ; the level of radioactivity in the normal tissues and organs was at or below that seen in the blood. The localization to xenografts was antigen specific and the accessible binding sites in 100-200-mg tumors appeared to be saturated at an antibody dose between 10 and 100 .mu.g. These findings suggest that the CYT-356 immunoconjugate may be useful in the diagnosis and therapy of prostate cancer.

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Immunoscintigraphy of prostatic cancer: Preliminary results with sup 1sup 1sup 1In-labeled monoclonal antibody 7E11-C5.3 (CYT-356)
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A phase 1 study was conducted with the investigational immunoscintigraphic agent, sup 1sup 1sup 1In-CYT-356, a radiolabeled, site-specific immunoconjugate of monoclonal antibody 7E11-C5.3, in 40

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Immunoscintigraphy of Prostatic Cancer: Preliminary Results With ^{111}In-Labeled Monoclonal Antibody 7E11-C5.3 (CYT-356)

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A phase I study was conducted with the investigational immunoscintigraphic agent, ^{111}In-CYT-356, a radiolabeled, site-specific immunoconjugate of monoclonal antibody 7E11-C5.3, in 40 patients with prostatic carcioma and known distant metastases. Each patient received a single intravenous infusion of CYT-356 (dose range, 0.1–5 mg) radiolabeled with approximately 5 mCi of ^{111}In. None of the patients experienced adverse reactions. One patient who received a 5-mg dose developed antibodies to the CYT-356 immunoconjugate. ^{111}In-CYT-356 immunoscintigraphy detected bony metastases in 21 of 38 patients (55%), including 12 of 14 (86%) receiving concomitant hormonal therapy, and soft tissue lesions in four of six patients (67%). Antibody imaging detected occult lesions in the bony pelvis and lumbar spine, which were confirmed by follow-up imaging tests, in one patient. Higher CYT-356 doses may clear the blood pool more slowly. These results suggest that ^{111}In-CYT-356 can be safely administered to patients with prostatic carcinoma and that further clinical investigation of this agent is warranted.

Key words: human antimouse antibodies, gamma scintigraphy, immunoconjugate

INTRODUCTION

Approximately one out of 11 men will develop adenocarcinoma of the prostate, making this disease the most common cancer among men in the United States [1]. Localized prostatic carcinoma is often asymptomatic, and over 50% of patients have nonlocalized disease at the time of their initial presentation [2,3]. The prognosis of

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patients with prostatic carcinoma and the selection of appropriate therapies is closely correlated with the clinical stage of disease, with the presence or absence of lymph node metastases being an important secondary prognostic indicator [2]. Because of the importance of accurate staging in the management of this disease, as well as the limitations of currently available nonsurgical diagnostic tests for the detection of the regional and metastatic spread of prostate cancer, surgical staging is often required [2].

Recent advances in monoclonal antibody technology have provided important new tools for basic research and clinical investigations in oncology. In patients with other types of cancer, immunoscintigraphy using radiolabeled monoclonal antibodies directed toward tumor-associated antigens has been found to identify tumor deposits not detected by other available radiographic techniques [4–7]. Thus, radioimmunodetection may prove particularly useful as a noninvasive diagnostic and staging modality for neoplastic diseases such as cancer of the prostate.

Several monoclonal antibodies directed toward antigens derived from the cytosol and membranes of prostatic cancer cells as well as antibodies to prostatic secretions have been investigated as potential radioimaging agents [8–10]. One such agent is 7E11-C5, a murine IgG1 monoclonal antibody. This antibody was produced by a hybridoma cell line originating from the fusion of murine myeloma cells with spleen cells of mice immunized with the human prostatic carcinoma cell line, LNCaP [11]. 7E11-C5 reacts with cytoplasmic membrane-rich fractions of LNCaP cells, but not with soluble cytosol or secretory glycoproteins, such as prostate-specific antigen or prostatic acid phosphatase. The target antigen recognized by monoclonal antibody 7E11-C5 is a 100 kD glycoprotein, which has been isolated from benign and malignant prostatic tissue, but has not been detected in any nonprostatic tissue examined [12].

Immunohistological evaluation of frozen tissue sections demonstrated that 7E11-C5 reacted with epithelial cells from prostatic carcinoma, benign prostatic hypertrophy, and, to a lesser degree, with normal prostate glands. Consistently negative results were obtained for immunospecific cell surface staining of numerous fresh frozen sections from a wide spectrum of human nonprostatic normal and malignant tissues [11]. Poorly defined staining of kidney tubules was noted in these studies.

Further nonclinical and clinical evaluations were conducted using a subclone of the 7E11-C5 parent hybridoma line. The subclone, designated 7E11-C5.3, was isolated, and the secreted monoclonal antibody was purified and then conjugated with the linker-chelator glycyl-tyrosyl-(N,ε-diethylenetriamine-pentaacetic acid)-lysine (GYK-DTPA) using the site-specific conjugation procedure developed by Rodwell et al. [13]. The resulting immunoconjugate, 7E11-C5.3-GYK-DTPA (CYT-356), exhibited reactivity similar to its parent cell line for both malignant and nonmalignant tissue [14].

Immunohistochemical evaluation of CYT-356 revealed a mosaic pattern of cytoplasmic staining with both skeletal and cardiac muscle in human, primate, and murine species. However, in vivo studies in mice and monkeys using ^{111}In-labeled CYT-356 demonstrated that the immunoconjugate did not accumulate in these tissues; thus, the pattern of intracellular binding would not be expected to interfere with the ability of this agent to image malignant prostatic disease. Moreover, toxicity studies conducted in rats and rabbits using doses over 1,700 times higher than the anticipated clinical imaging dose (approximately 0.5 mg) showed no biochemical or histological

evidence of cardiomyopathy or myositis. Finally, specific localization of ^{111}In-labeled CYT-356 was observed in LNCaP human prostate carcinoma xenografts, with tumor-to-blood ratios of 3:1; tumor concentrations of the radiolabeled immunoconjugate averaged 34% of the injected dose/gram [14].

On the basis of the favorable pattern of immunohistologic reactivity and the in vivo specificity and toxicity data, clinical investigations of ^{111}In-CYT-356 were initiated in patients with prostatic carcinoma. We present here the results of a phase 1 multicenter clinical trial of this radiolabeled antibody conjugate in patients with a tissue diagnosis of prostate cancer and documented extrapelvic metastases.

MATERIALS AND METHODS

The objectives of this phase 1 study were to evaluate the safety, pharmacokinetics, and development of human antimouse antibodies (HAMA) following a single intravenous infusion of various doses of ^{111}In-CYT-356. A secondary objective was to obtain a preliminary assessment of the ability of ^{111}In-CYT-356 immunoscintigraphy to detect metastatic prostatic carcinoma lesions. This dose-ranging trial was conducted at seven clinical study sites (see Acknowledgments). The protocol was approved by the institutional review boards at each participating center, and each patient enrolled in the study granted written informed consent.

Patient Population

Eligible patients were adult men with a tissue diagnosis of prostate cancer and extrapelvic metastases documented by a standard diagnostic and staging procedure, such as radionuclide bone scan, chest x-ray, computed tomographic (CT) scan, magnetic resonance imaging (MRI), etc. None of the patients were surgical candidates. With the exception of hormonal treatments, which were permitted during the study period, patients could not receive antitumor adjuvant therapy for at least 3 weeks prior to study entry. In addition, patients must have recovered from any toxicity associated with prior antitumor therapy, and were required to have a Karnofsky performance status score of at least 60% and an expected survival of at least 2 months.

As this was the first clinical investigation using ^{111}In-CYT-356, patients who had received a previous administration of a murine antibody, those with second primary malignancies (except in situ carcinomas) or known brain metastases, and patients with abnormal hematologic status, renal function, or liver function were excluded from participation. Also excluded were patients with other serious illnesses or conditions that could have precluded the completion of the required study procedures.

A total of 40 men were enrolled in this study. All but two of the patients were white, and the population had a mean age of 70 years. The demographic characteristics of the patients are summarized in Table 1 for the entire study population and for each ^{111}In-CYT-356 dose group.

Preparation of ^{111}In-CYT-356

Monoclonal antibody 7E11-C5.3 was produced in cell culture by Invitron Corporation (St. Louis, MO), and the linker-chelator, GYK-DTPA, was produced by JBL Scientific, Inc. (San Luis Obispo, CA) using a procedure developed by CYTOGEN Corporation [15]. The oligosaccharide moiety of the antibody was site-specif-

TABLE I. Patient Demographic Characteristics by ^{111}In-CYT-356 Dose Group

Attribute	Administered CYT-356 Dose (mg)					All doses (total)
	0.1	0.2	0.5	1	5	
No. of Patients	4	11	11	7	7	40
Age (yr)						
mean	71	68	70	68	72	70
range	66–78	57–78	64–84	58–80	60–81	57–84
Race (no.)						
White	4	10	10	7	7	38
Black	0	1	1	0	0	2
^{111}In Dose (mCi)						
mean	5.2	4.1	5.5	5.4	5.6	5.1
range	4.9–5.4	3.4–4.9	4.7–6.5	5.1–6.1	5.1–6.1	3.4–6.5
Prior antitumor therapies (n)						
hormonal therapy	1	8	5	3	3	20
chemotherapy/ immunotherapy	0	0	0	1	0	1
radiation therapy	1	5	6	3	3	18
Concurrent						
hormonal therapy (n)	0	7	3	2	3	15

ically conjugated with GYK-DTPA, as described previously [13]. Single doses of the resulting immunoconjugate, CYT-356 (7E11-C5.3-GYK-DTPA; CYTOGEN Corporation, Princeton, NJ), were aseptically filled into individual vials and provided to each study site.

Patient doses of ^{111}In-CYT-356 were prepared by the radiopharmacy staff at each study center. $^{111}InCl_3$ (Amersham Corporation, Arlington Heights, IL) was buffered and added to the vial containing the CYT-356 dose. The solution was mixed and incubated at ambient temperature for 30 min. The radiolabeled CYT-356 was filtered through a 0.22-μm filter, tested for free ^{111}In using an instant thin layer chromatographic procedure [16], and delivered to the site of administration.

Study Plan

At study entry, a medical history was obtained, and a physical examination, including an electrocardiogram, was performed. Baseline laboratory evaluations included standard serum chemistry and hematology tests and routine urinalysis. The results of all diagnostic and staging evaluations made within 4 weeks of study entry were recorded; the protocol required that each patient have a chest x-ray and a radionuclide bone scan performed during this period.

HAMA titers were evaluated using the ImmuSTRIP® HAMA Test System (Immunomedics, Warren, NJ) as described previously [7]. This system employs a direct enzyme-linked immunosorbent assay (ELISA) for the detection and quantitation of human antibodies to mouse IgG; serum HAMA titers > 0.4 μg/mL are considered positive by this method. In addition, a CYT-356-specific ELISA (CYTOGEN Corporation, Princeton, NJ) was used to measure serum titers of anti-CYT-356 antibodies.

After the completion of the baseline evaluations, eligible patients received a single dose (either 0.1, 0.2, 0.5, 1, or 5 mg) of CYT-356 radiolabeled with approximately 5 mCi of ^{111}In and administered as a 5-min intravenous infusion. Table I lists

the numbers of patients who received each CYT-356 dose and summarizes the mean ^{111}In activities of each dose group. Vital signs were monitored before and at selected intervals during the 2 hr after the ^{111}In-CYT-356 infusion. Laboratory evaluations were repeated 3 days postinfusion and a final on-study physical examination was performed 4 weeks after ^{111}In-CYT-356 administration. Blood samples for repeat HAMA titers were collected at selected intervals through 24 weeks postinfusion.

Pharmacokinetic Determinations

The pharmacokinetic profile of ^{111}In-CYT-356 was assessed in a subgroup of patients at selected study sites. Serial blood samples were obtained prior to ^{111}In-CYT-356 infusion and at 5, 15, 30, 60, 90, 120, and 240 min postinfusion. Additional blood samples were obtained at the time of each gamma camera imaging session. Complete urine collections were obtained postinfusion over the following periods: 0–2 h, 2–24 h, and at 24-hr intervals up to the final imaging session. Duplicate aliquots of blood, serum, and urine were counted in a gamma well counter adjusted for an ^{111}In window. Standards prepared from the administered ^{111}In-CYT-356 doses were counted at the same time and used to correct the ^{111}In activities of the samples for radioactive decay. Blood and serum concentration-vs.-time data were plotted and exponential functions were fit to the data in order to calculate standard pharmacokinetic parameter estimates for each patient. Cumulative urinary excretion plots were generated for each patient and fitted to a single exponential function to determine the renal clearance rate constant of the ^{111}In-labeled CYT-356.

Imaging Protocol

Planar imaging. Prior to study initiation, each investigator performed the following quality control procedures: intrinsic ^{111}In flood and bar phantom (minimum of two million counts), extrinsic flood (medium energy collimator on) using a ^{57}Co or ^{111}In flood source. The intrinsic ^{111}In flood and bar phantom images were acquired prior to each imaging session, and the extrinsic flood image was acquired weekly throughout the study period.

SPECT imaging. Quality control of the single photon emission tomographic (SPECT) imaging system included uniformity, axis of rotation, and patient motion detection (sinogram display). An extrinsic uniformity correction flood (30–120 million counts) was acquired using ^{111}In for SPECT reconstruction if a fillable flood phantom was available. Alternatively, a ^{57}Co disc source was used.

Imaging plan. All patients were imaged using a large field-of-view gamma camera equipped with a parallel hole medium energy collimator and dual pulse-height analyzer. Energy settings had 20% symmetrical windows centered at 172 KeV and 247 KeV. Planar gamma camera imaging was initially performed on three occasions at 1, 2, and 3 days postinfusion. Because of increased blood pool background in the day 1 images, later patients enrolled in the study were imaged between days 2 and 5. Anterior and posterior views of the pelvis, abdomen, and thorax were acquired at each imaging session (7.5 min/view); additional images were obtained of other regions known or suspected to be involved with tumor. On the third imaging session, 5-min views of the humeri, femurs, and skull were obtained for each patient.

SPECT imaging of the pelvis and other regions suspicious for tumor was performed on day 3 postinfusion.

Evaluati n f Diagnostic Imaging Data

The planar and SPECT images were reviewed by the nuclear medicine physicians at each clinical study site. Copies of their reports describing the results of ^{111}In-CYT-356 immunoscintigraphy were included in the patients' case report forms. The antibody imaging findings for each patient were tabulated; abnormal radiolocalizations compatible with tumor were categorized as either bony lesions or soft tissue lesions. Confirmation of each such lesion detected by antibody scanning was sought based on the results of other standard imaging tests (e.g., radionuclide bone scan) and, where available, tissue biopsy findings. Data from follow-up imaging studies conducted to evaluate abnormal antibody imaging findings also were recorded. Also noted were lesions detected by other imaging modalities but not detected by immunoscintigraphy.

RESULTS

Safety Results

None of the patients infused with ^{111}In-CYT-356 experienced any adverse reactions related to the study agent. In addition, no clinically significant changes in vital signs were observed following the infusions. Finally, there were no clinically abnormal laboratory findings or changes from preinfusion values related to ^{111}In-CYT-356 administration.

HAMA Titers

None of the patients developed detectable titers of anti-murine IgG antibodies postinfusion. As measured by a commercially available ELISA assay, one patient was noted to have a positive HAMA titer (0.81 μg/mL) prior to receiving the ^{111}In-CYT-356 infusion. This patient's titer was later determined to represent a false positive value due to the presence of rheumatoid factor in his serum. Postinfusion titers for this patient were unchanged from the preinfusion level through 2 months after administration of ^{111}In-CYT-356.

None of the patients developed HAMA as measured by the ImmuSTRIP® HAMA Test System, which measures reactivity to the constant region of mouse immunoglobulin. However, one patient developed a low HAMA titer in a specific CYT-356 ELISA test system, which measures reactivity to both the constant and variable regions of the infused monoclonal antibody. This patient had received an infusion of the highest (5 mg) dose of radiolabeled CYT-356.

Imaging Results

Table II summarizes the results of ^{111}In-CYT-356 immunoscintigraphy for the detection of prostatic carcinoma lesions. The results have been presented for each ^{111}In-CYT-356 dose group; tumor lesions have been separated into bony metastases and soft tissue tumor lesions. As shown, 38 of the 40 patients had bony metastases, as documented by standard radiographic modalities. Overall, antibody imaging detected known bony lesions in 55% of patients. The sensitivity of antibody imaging for detection of these metastases was lowest among the patients who received 0.1-mg doses of radiolabeled CYT-356 (25%; 1/4) and highest among the 0.2-mg dose group (90%; 9/10); whereas CYT-356 doses $\geq$ 0.5 mg were associated with a sensitivity of

TABLE II. Results of ^{111}In-CYT-356 Antibody Imaging (MAb) f r the Detection of Bony and Soft Tissue Lesions of Prostatic Carcinoma

		Bony metastases: No. (%) of patients				
		Known disease		Additional disease detected by MAb		
CYT-356 dose (mg)	No. of patients	Total	Detected by MAb	Total	Evaluated by other tests	Confirmed by other tests
0.1	4	4	1 (25%)	1	1	0
0.2	11	10	9 (90%)	1	1	1
0.5	11	10	5 (50%)	0	0	0
1	7	7	3 (43%)	1	1	0
5	7	7	3 (43%)	0	0	0
Total	40	38	21 (55%)	3	3	1
		Soft tissue lesions: No. (%) of patients				
		Known disease		Additional disease detected by MAb		
CYT-356 dose (mg)	No. of patients	Total	Detected by MAb	Total	Evaluated by other tests	Confirmed by other tests
0.1	4	1	1 (100%)	2	0	0
0.2	11	1	1 (100%)	7	2	0
0.5	11	4	2 (50%)	1	0	0
1	7	0	0	2	0	0
5	7	0	0	1	0	0
Total	40	6	4 (67%)	13	2	0

46% (11/24). Fourteen of the 15 patients who were receiving concomitant hormonal therapy had documented bony disease; antibody imaging localized known bony lesions in 12 of these 14 patients (86%).

In most cases where antibody imaging detected known bony metastases, immunoscintigraphy localized only a subset of the lesions detected by radionuclide bone scanning. Overall, 33 patients had one or more bony lesions documented by bone scan that were not detected by antibody imaging. Since no attempt was made to biopsy these lesions, it is unknown whether the bone scan positive/antibody scan negative findings represented active tumor lesions or inactive tumor or inflammatory lesions with a surrounding area of reactive bone. In one case (Fig. 1), ^{111}In-CYT-356 immunoscintigraphy detected occult bony lesions in the lumbar spine and the bony pelvis, which were not detected on bone scan and which were confirmed by subsequent MRI studies. Additional occult bony lesions detected in two other patients were not confirmed by other diagnostic imaging modalities. These results suggest that antibody imaging has the potential to detect occult bony lesions; however, a definitive determination of the sensitivity and specificity of this procedure will require confirmation by biopsy.

As shown in Table II, six patients had documented soft tissue tumor lesions. ^{111}In-CYT-356 immunoscintigraphy detected soft tissue disease in four of these six patients (67% sensitivity); the lesions detected in these four patients included lung

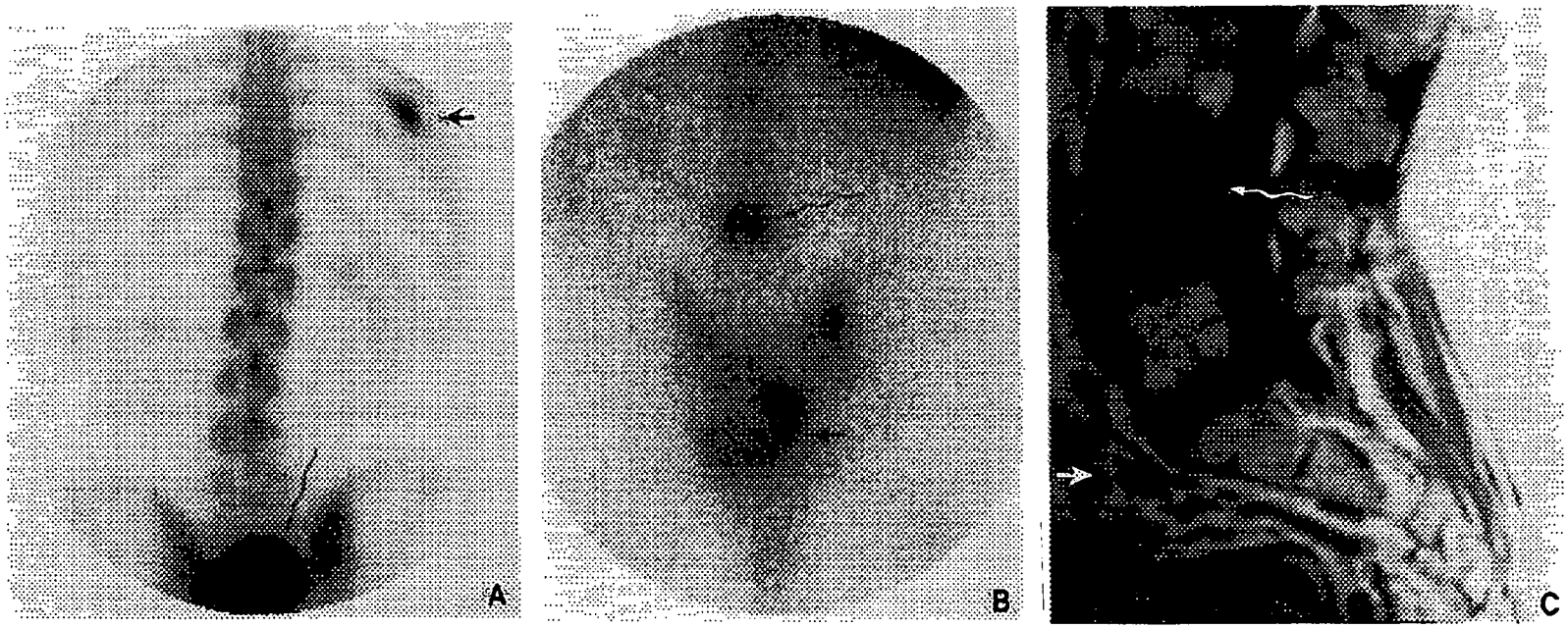

Fig. 1. Diagnostic evaluations for a 57-year-old man with a 10-year history of prostatic carcinoma. Initial study was a radionuclide bone scan (A), showing intense tracer accumulation in a posterior right rib (straight arrow), which was consistent with metastasis, and intense activity over a distended urinary bladder (curved arrow), which hindered evaluation of the pelvis. Gamma camera imaging was then performed; a posterior view of the pelvis (B) acquired 72 hours postinfusion of ^{111}In-CYT-356 showed radiolocalizations consistent with metastases in the lumbar spine (curved arrow) and the bony pelvis (straight arrow). The antibody scan findings in the lumbar spine (curved arrow) and the pelvis (straight arrow) were confirmed by a subsequent magnetic resonance imaging study (C).

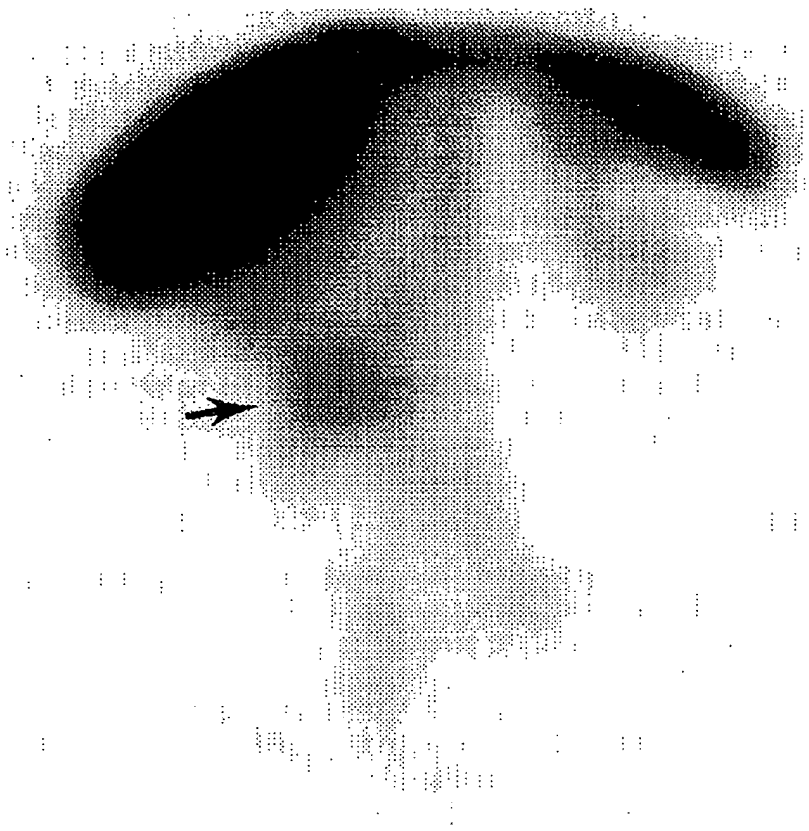

Fig. 2. Coronal tomographic image of a 66-year-old man with a 13-year history of prostatic carcinoma. The radiolocalization in the retroperitoneal lymph nodes (straight arrow), which was also detected by computed tomography, was confirmed by tissue biopsy to be a metastatic lesion. The diffuse radiolocalization in the upper part of the image represents nonspecific uptake of the ^{111}In-labeled immunoconjugate by the liver and spleen.

metastases (n = 2), retroperitoneal adenopathy (n = 1), mediastinal metastases (n = 1), and supraclavicular lymphadenopathy (n = 1). The antibody scan of one of these patients, who was found to have retroperitoneal lymphadenopathy which was confirmed by tissue biopsy, is presented in Figure 2. One of these four patients, in whom antibody imaging detected disease in the left lung, had an additional lesion in the right lung, which was not identified on the antibody scans. Antibody imaging also failed to detect periaortic lymphadenopathy in one patient and tumor in the prostate or periprostatic tissue in another patient. Finally, ^{111}In-CYT-356 immunoscintigraphy detected additional soft tissue lesions in 13 patients. Follow-up imaging studies, which failed to confirm the antibody scan findings, were performed in two of these cases: positive localization in the prostatic or periprostatic tissue of one patient was not confirmed by CT imaging, and radiolocalization in soft tissue dorsal to the spine was noted at the site of a previous radiation port in the other patient. Confirmatory testing was not performed for the remaining 11 patients to evaluate the soft tissue sites localized by antibody imaging; the majority of these sites involved either pelvic or abdominal lymph nodes or prostatic or periprostatic tissues.

Pharmacokinetic Results

To date, pharmacokinetic evaluations have been completed for nine patients; of these, five patients received 0.2-mg doses, and the two patients each received ^{111}In-CYT-356 doses of 0.5 and 5 mg. For the five patients administered 0.2-mg infusions of radiolabeled CYT-356, serum pharmacokinetics (mean ± SD) of the ^{111}In-labeled immunoconjugate were best described by a one-compartment pharmacokinetic model and were characterized by a small volume of distribution (2485 ± 447 mL) and a slow serum clearance rate (44.9 ± 15.5 mL/hr), which resulted in a long serum half-life (40.4 ± 7.8 hr). The rate constant for urinary excretion averaged 0.007 ± 0.002 hr^{-1}. Although data from additional patients are required before definitive comparisons can be made, the results to date suggest that the volume of distribution and serum clearance rate of the ^{111}In-labeled CYT-356 appear to decrease and the serum half-life appears to increase as the CYT-356 dose increases.

DISCUSSION

The results of this phase 1 study indicate that CYT-356, in doses ranging from 0.1 to 5 mg, radiolabeled with approximately 5 mCi of ^{111}In, can be safely administered to patients with cancer of the prostate. None of the 40 patients enrolled in this study experienced clinical symptoms, allergic-type reactions, vital sign changes, or clinically abnormal laboratory findings after receiving a single intravenous infusion of the study agent. An encouraging and somewhat surprising finding was the absence of HAMA development to mouse IgG after ^{111}In-CYT-356 administration. Moreover, only one patient developed HAMA to CYT-356 postinfusion, and this patient received the highest (5 mg) dose of radiolabeled CYT-356. In general, administration of intact murine antibodies induces a positive HAMA response in a certain subset of patients [8,17,18]. For example, administration of 40 and 80 mg doses of PAY-276, an antibody directed against prostatic acid phosphatase, produced a positive HAMA response in eight of 16 (50%) patients [8]. Although the clinical importance of HAMA, in terms of the safety and efficacy of repeated administrations of murine proteins, remains to be determined, immunoscintigraphic agents with low immunogenicity have potential advantages over more immunogenic molecules [17,18].

The preliminary efficacy results from this initial ^{111}In-CYT-356 investigation have demonstrated that the radiolabeled immunoconjugate localizes to bony and soft tissue prostatic carcinoma lesions. Overall, ^{111}In-CYT-356 immunoscintigraphy detected bony metastases in 21 of 38 patients (55%) with documented lesions. Moreover, antibody imaging detected occult bony disease, which was not detected by radionuclide bone scanning, in one patient; these lesions in the lumbar spine and bony pelvis were subsequently confirmed by other imaging tests. It is also interesting to note that the sensitivity of ^{111}In-CYT-356 immunoscintigraphy for bone lesions was not reduced in patients receiving hormonal therapies; in fact, sensitivity was high in this subgroup of patients (86%; 12 of 14 patients).

In this series, antibody imaging detected only a subset of the bony metastases identified by bone scanning. Because bone scanning is known to be sensitive but not highly specific for the detection of bony metastases [19,20], and because no pathologic verification was performed for the detected lesions, the significance of these findings is uncertain. In fact, some of the lesions detected by bone scanning may

represent inflammatory processes, arthritic changes, or healed tumor lesions. Moreover, published findings with radiolabeled antibodies directed against prostatic acid phosphatase [9,21,22] or prostate specific antigen [10,23] indicate that these agents also identified only a subset of the bony lesions detected by bone scanning. Finally, in one of these studies [23], the monoclonal antibody scan findings correlated more closely than the bone scan results with the patients' clinical status over a 2-year follow-up period.

Additional studies with ^{111}In-CYT-356 will be required to demonstrate whether this agent is highly specific for the detection of bony metastases and to determine if antibody imaging is useful to clarify equivocal bone scan findings. In the present phase 1 study, there were some cases in which ^{111}In-CYT-356 immunoscintigraphy did provide useful information concerning bony lesions, in particular the case in which it detected occult bony disease.

The results also indicate that antibody imaging with ^{111}In-CYT-356 detected documented soft tissue tumors in four of six patients. Although these findings are encouraging, they can only be viewed as preliminary observations because of the small number of patients with documented soft tissue disease and the lack of pathological confirmation for most of these lesions. Moreover, no follow-up imaging studies were performed to evaluate the majority of additional soft tissue lesions (11 of 13) identified by antibody imaging. Since most of these lesions were located in areas commonly involved with the spread of prostatic cancer (i.e., pelvic or abdominal lymph nodes, prostatic or periprostatic tissues), it is likely that at least some of these findings correspond to actual tumor lesions. These preliminary data suggest that ^{111}In-CYT-356 immunoscintigraphy may be useful for the detection of local-regional disease and metastatic tumor deposits in patients with prostate carcinoma. These findings also indicate that further investigations of the immunoconjugate are indicated in prostate cancer patients scheduled to undergo staging lymphadenectomy. Studies in this patient population would allow surgical and pathological confirmation of the immunoscintigraphic findings.

One of the objectives of this phase 1 study was to evaluate various doses of ^{111}In-labeled CYT-356 in order to discover any dose-response trends. The preliminary pharmacokinetic data suggest that the serum half-life may increase with administered CYT-356 dose. As noted, this finding is consistent with the observation that the blood pool clearance of the radiolabeled immunoconjugate appeared to be slower at the higher dose levels. These somewhat anecdotal results are especially interesting in terms of the imaging efficacy data. Although the numbers of patients administered each CYT-356 dose level are small, the per-patient sensitivity for bony metastases was highest among the patients who received one of the lower doses (0.2 mg) of radiolabeled CYT-356. If these imaging results are confirmed in a larger sample, and if the pharmacokinetic data show that a more rapid blood pool clearance allows for earlier acquisition of diagnostic images, lower CYT-356 doses may actually be preferable for future studies with ^{111}In-CYT-356.

In contrast to the results with ^{111}In-CYT-356, positive dose-response trends have been observed with the antiprostatic acid phosphatase antibody PAY-276 [22]. With PAY-276, the per- lesion sensitivity of the antibody scans increased from 8% following administration of a 5 mg dose of ^{111}In-labeled PAY-276 to 76% after an 80 mg dose. However, positive HAMA titers developed in 50% of the patients administered the higher doses. When compared with the results of radioimmunodetection

studies using PAY-276 [9,22] and other antibodies [8,10,21,23], our preliminary findings with ^{111}In-CYT-356 are particularly encouraging because of the relatively high tumor detection rates associated with administration of low immunoconjugate doses, which were also associated with a low frequency of HAMA development.

CONCLUSIONS

The results of the initial clinical investigation of ^{111}In-CYT-356 have demonstrated that this agent can be safely administered to patients with prostatic carcinoma. In addition, ^{111}In-CYT-356 immunoscintigraphy detected soft tissue and bony lesions, including occult disease, in this patient population, suggesting the potential utility of this agent in improving the accuracy of staging of prostate cancer patients. Further studies are required to determine the effect of CYT-356 dose on efficacy, safety, and biodistribution of this agent. Because of these encouraging preliminary findings, studies have been designed to provide a definitive evaluation of the imaging performance of ^{111}In-CYT-356 in patients scheduled to undergo staging lymphadenectomy. Additional trials also will determine the clinical utility of this immunoscintigraphic agent in the context of the standard diagnostic procedures used for prostate cancer, both as a comparative and complementary imaging modality.

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REFERENCES

1. "Cancer Facts." Atlanta: American Cancer Society, Inc., 1990, p. 12.
2. Perez CA, Fair WR, Ihde DC, Labrie F: Cancer of the prostate. In: DeVita VT, Helman S, Rosenberg SA (eds): "Cancer, Principles and Practice of Oncology." Philadelphia: J.B. Lippincott Company, 1989, pp 929–964.
3. Murphy GP, Natarajan N, Pontes JE, et al.: The national survey of prostate cancer in the United States by the American College of Surgeons. J Urol 127:928–934, 1982.
4. Larson SM: Lymphoma, melanoma, colon cancer: Diagnosis and treatment with radiolabeled monoclonal antibodies. Radiology 165:297–304, 1987.
5. Beatty JD, Williams LE, Yamauchi D, et al.: Presurgical imaging with indium-labeled anticarcinoembryonic antigen for colon cancer staging. Cancer Res (suppl) 50:922s–926s, 1990.
6. Salk D and the Multicenter Study Group: Technetium-labeled monoclonal antibodies for imaging metastic melanoma: Results of a multicenter clinical study. Semin Oncol 15:608–618, 1988.
7. Maguire RT, Schmelter RF, Pascucci VL, Conklin JJ: Immunoscintigraphy of colorectal adenocarcinoma: Results with site-specifically radiolabeled B72.3 (^{111}In-CYT-103). Antibody, immunoconjugates, and Radiopharmaceuticals 2:257–269, 1989.
8. Babaian RJ, Lamki LM: Radioimmunoscintigraphy of prostate cancer. Semin Nucl Med 19:309–321, 1989.
9. Halpern SE, Haindl W, Beauregard J, et al.: Scintigraphy with In-111-labeled monoclonal antitumor antibodies: Kinetics, biodistribution, and tumor detection. Radiology 168:529–536, 1988.
10. Larson A, Arnberg H, Maripuu E, Nilsson S: Radioimmunodetection of prostatic cancer with

^{125}I-labelled antibody against prostatic specific antigen. Scand J Urol Nephrol 110 (Suppl):149–153, 1988.
11. Horoszewicz JS, Kawinski E, Murphy GP: Monoclonal antibodies to a new antigenic marker in epithelial prostatic cells and serum of prostatic cancer patients. Anticancer Res 7:927–936, 1987.
12. Wright GL, Jr, Feng Q, Beckett ML, Lopes D, Gilman SC: Characterization of a new prostate carcinoma-associated marker: 7E11-C5. Antibody, Immunoconjugates, and Radiopharmaceuticals 3:39, 1990.
13. Rodwell JD, Alvarez VL, Lee C, et al.: Site-specific covalent modification of monoclonal antibodies: In vitro and in vivo evaluations. Proc Natl Acad Sci USA 83:2632–2636, 1986.
14. Rosenstraus MJ, Davis WJ, Lopes AD, D'Aleo C, Gilman S: In vitro and in vivo reactivity of anti-prostate monoclonal antibody immunoconjugate 7E11-C5.3-GYK-DTPA. Antibody, Immunoconjugates, and Radiopharmaceuticals 3:54, 1990.
15. Alvarez VL, Lopes AD, Lee C, Coughlin DJ, Rodwell JD, McKearn TJ: Site-specific modification of monoclonal antibodies. In: Rodwell JD (ed): "Antibody-Mediated Delivery Systems." New York: Marcel Dekker, Inc., 1988, pp 283–315.
16. Zimmer AM, Kazikiewicz JM, Spies SM, Rosen ST: Rapid miniaturized chromatography for ^{111}In-labeled monoclonal antibodies: Comparison to size exclusion high performance chromatography. Nucl Med Biol 15:717–720, 1988.
17. Reynolds JC, Del Vecchio S, Sakahara H, et al.: Anti-murine antibody response to mouse monoclonal antibodies: Clinical findings and implications. Nucl Med Biol 16:121–125, 1989.
18. Perkins AC, Pimm MV, Powell MC: The implications of patient antibody response for the clinical usefulness of immunoscintigraphy. Nucl Med Commun 9:273–282, 1988.
19. Spirnak JP, Resnick MI: Clinical staging of prostatic cancer: New modalities. Urol Clin North Am 11:221–235, 1984.
20. Jacobson AF, Stomper PC, Cronin EB, Kaplan WD: Bone scans with one or two new abnormalities in cancer patients with no known metastases: Reliability of interpretation of initial correlative radiographs. Radiology 174:503–507, 1990.
21. Goldenberg DM, DeLand FH: Clinical studies of prostatic cancer imaging with radiolabeled antibodies against prostatic acid phosphatase. Urol Clin North Am 11:277–281, 1984.
22. Babaian RJ, Murray JL, Lamki LM, et al.: Radioimmunological imaging of metastatic prostatic cancer with ^{111}Indium-labeled monoclonal antibody PAY 276. J Urol 137:439–443, 1987.
23. Meyers FJ, Denardo SJ, Macey D, White RD, Unger M: Development of monoclonal antibody imaging of metastatic prostatic carcinoma. Prostate 14:209–220, 1989.